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Neuropharmacological profile of EMD 57445, a σ receptor ligand with potential antipsychotic activity

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Abstract

EMD 57445 ((*S*)-(−)-[4-hydroxy-4-(3,4-benzodioxol-5-yl)-piperidin-1-ylmethyl]-3-(4-methoxyphenyl)-oxazolidin-2-one) is a new σ receptor ligand with only marginal affinity for many other (including dopamine) receptors. In the present study, central, particularly neuroleptic-like, effects of this compound were evaluated and compared with those of another σ receptor ligand, rimcazole. EMD 57445 decreased locomotor activity in rats and mice. The amphetamine-induced locomotor hyperactivity and stereotypy were reduced by EMD 57445. The drug was able to inhibit the behavioural effects induced by apomorphine, i.e., the locomotor hyperactivity, stereotypy and aggression in rats, as well as climbing in mice. The hyperlocomotion induced by quinpirole (a dopamine $D_{2/3}$ receptor agonist) and the grooming induced by SKF 38393 (a dopamine D_1 receptor agonist) were decreased by EMD 57445. The behavioural stimulation evoked in rats by non-competitive (MK-801, (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(*a,b*)-cyclohepten-5,10-imine hydrogen maleate) or competitive (CGP 37849, D,L-E-amino-4-methyl-5-phosphono-3-pentenoic acid) NMDA receptor antagonists was also inhibited. EMD 57445 decreased the cocaine-, morphine- or caffeine-induced locomotor hyperactivity in rats or mice. It neither induced catalepsy nor increased muscle tone in rats. Rimcazole had somewhat different effects: it increased the amphetamine stereotypy as well as the amphetamine-, quinpirole- and cocaine-induced locomotor hyperactivity in rats. The results indicate that EMD 57445 shows functional antidopaminergic activity and may be useful as an antipsychotic drug devoid of extrapyramidal side-effects.

Keywords: σ Receptor ligand; EMD 57445; Rimcazole; Central effect; Behavioral model

1. Introduction

σ Receptors were first proposed by Martin et al. (1976) to explain the pharmacological effects of benzomorphans, such as *N*-allylnormetazocine ((+)-SKF 10,047) and pentazocine. They were first defined as an opioid receptor subtype and were later thought to be identical to phencyclidine binding sites (Zukin and Zukin, 1981; Mendelsohn et al., 1985). Several lines of evidence now indicate the existence of distinct σ and phencyclidine binding sites (Tam, 1983; Gundlach et al., 1985; Quirion et al., 1987). In addition, a high affinity of some neuroleptics, e.g., haloperidol, perphenazine, fluphenazine, chloropromazine, tioridazine, pimozide and (−)-butaclamol, for σ receptors (or binding sites) has been demonstrated (e.g., Tam and Cook, 1984; Itzhak, 1988). The latter fact and other findings (see Walker et al., 1990) lead to the hypothesis that σ receptors may be involved in the pathogenesis of schizophrenia and/or in the mechanism of action of antipsychotic drugs. Some σ receptor ligands have already been found, e.g., DTG (1,3-di-*o*-tolylguanidine), BMY 14802, rimcazole, remoxipride and their potential antipsychotic (antidopaminergic, neuroleptic-like, without inducing catalepsy) activity has been described (Ferris et al., 1982, 1986a,b; Ögren et al., 1984; Hall et al., 1986; Weber et al., 1986; Taylor and Dekleva, 1987; Largent et al., 1988; Maj et al., 1993a). First clinical trials demonstrated their moderate antipsychotic activity in schizophrenia (Davidson et al., 1982; Guy et al., 1983; Chouinard and Annable, 1984; Schwarcz et al., 1985). However, it is still unclear which effects are induced by the agonistic or by the antagonistic action on σ receptors. This could result from insufficient affinity and selectivity of known agents for σ binding sites. Recently, a new substance, EMD 57445 ((*S*)-(−)-[4-hydroxy-4-(3,4-benzodioxol-5-yl)-piperidin-1-ylmethyl]-3-(4-methoxyphenyl)-oxazolidin-2-one), has been developed (Bartoszyk et al., 1992, 1996), which shows high affinity for σ receptors (but not for any other

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receptors, including dopamine) and potent antidopaminergic activity. The drug antagonizes the apomorphine stereotypy and climbing, inhibits avoidance behaviour, shows only marginal cataleptic activity at very high doses and does not induce supersensitization of dopamine receptors after chronic treatment (Bartoszyk et al., 1992, 1995, 1996). We now studied the neuropharmacological profile of EMD 57445, particularly with respect to its potential antipsychotic activity. Another σ receptor ligand, rimcazole (Ferris et al., 1982, 1986a,b), was used for comparison. Some of our results obtained with rimcazole have been presented earlier (Maj et al., 1993a,1994). The results for EMD 57445 were presented at the VIII Congress of the European College of Neuropsychopharmacology (Maj et al., 1995).

2. Materials and methods

The experiments were carried out on non-fasted male Wistar rats (220–270 g) or male Albino Swiss mice (22–26 g), housed in groups of 10 or 20, respectively, on a natural day-night cycle, with free access to food and water. All the substances were dissolved in distilled water. Spiperone and caffeine were dissolved in a water solution of citric acid and adjusted to pH $\approx$ 7 with 0.1 M NaOH. EMD 57445 was given orally (p.o.) in doses of 1, 3 and 10 mg/kg. Amphetamine, apomorphine and quinpirole were given subcutaneously (s.c.), all the other substances were administered intraperitoneally (i.p.). Control animals received vehicle according to the same schedule. Each animal was used for a single experiment and a single experimental dose.

2.1. Locomotor activity in rats and mice

Locomotor activity was measured in photoresistor actometers (2 light beams), in which the animals were placed individually. EMD 57445 (1, 3 and 10 mg/kg) or rimcazole (10, 20 and 40 mg/kg in rats, and 5, 10 and 20 mg/kg in mice) was given 1 h before the test. Locomotor activity was measured for 1 h.

The following central stimulants were used in the locomotor activity test:

- apomorphine (0.3 mg/kg given 15 min before the test);
- D-amphetamine (0.3 mg/kg 30 min before the test);
- quinpirole (0.3 mg/kg 30 min before the test);
- CGP 37849 (20 mg/kg 30 min before the test) or MK-801 (0.2 mg/kg 30 min before the test);
- cocaine (20 mg/kg in rats 15 min before the test, and 16 mg/kg in mice 30 min before the test);
- morphine (20 mg/kg in mice 30 min before the test);
- caffeine (20 mg/kg 30 min before the test).

Locomotor activity was measured for 1 h, except for the quinpirole-induced hyperactivity, which was recorded for 2 h. Each group consisted of 8 rats or 10 mice.

2.2. D-Amphetamine- and apomorphine-induced stereotypy

The animals treated with EMD 57445 (1, 3 and 10 mg/kg) or rimcazole (5, 10 and 20 mg/kg) were placed individually in wire cages; 30 min later, they were injected with either amphetamine (2.5 mg/kg) or apomorphine (0.5 mg/kg). The intensity of stereotyped activities was assessed according to an arbitrary 4-point scale (0, normal; 1, periodic sniffing; 2, continuous sniffing; 3, licking; and 4, gnawing and biting). The stereotypy was evaluated at 30-min intervals for 3 h in the amphetamine test, or at 10-min intervals for 1 h in the apomorphine test. Each group consisted of 8 animals.

2.3. Apomorphine-induced aggression in rats

1 h after the injection of EMD 57445 (1, 3 and 10 mg/kg) or rimcazole (5, 10 and 20 mg/kg), apomorphine (10 mg/kg) was given to rats and they were then paired in wire cages (20 × 20 × 23 cm). Fighting behaviour was defined as both rats (in a pairs) assuming a mutual upright posture, standing on their hindlegs, or one animal forcing its partner to assume a different pattern of submissive posture. Groups consisted of 10 rats each.

2.4. Apomorphine-induced climbing in mice

For observation, the mice were placed in individual cylindrical cages (12 cm in diameter, 14 cm high) with walls made of vertical metal bars (2 mm in diameter, 1 cm apart). After a 0.5-h adaptation period, the mice were given EMD 57445 (1, 3 and 10 mg/kg) or rimcazole (5, 10 and 20 mg/kg) and, 1 h later, apomorphine (0.75 mg/kg). Immediately afterwards, behaviour was scored as follows: 4 paws on the floor, 0 point; forefeet held on the wall, 1 point; 4-paws-hold on the wall, 2 points (according to Protais et al., 1976). Groups consisted of 10 mice each.

2.5. SKF 38393-induced grooming in rats

EMD 57445 (1, 3 and 10 mg/kg) or rimcazole (5, 10 and 20 mg/kg) was administered 45 min before SKF 38393 (10 mg/kg). 15 min later, the animals were placed individually in the wire cages and the total time of grooming was measured for 15 min. Groups consisted of 8 rats each.

2.6. Catalepsy in rats

Catalepsy was evaluated according to the slightly modified method of Delini-Stula and Morpurgo (1968). Each rat was tested with respect to its right and left front paws, which were put on columns 3 and 9 cm high; the score was 1 or 2, respectively (max. 6 points for the right and left paws), if a rat maintained an abnormal body posture for more than 10 s. EMD 57445 (10 and 20 mg/kg) or

rimcazole (20 and 40 mg/kg) was given 30 min before the test. In a separate experiment, EMD 57445 (1 and 10 mg/kg) or rimcazole (5 and 10 mg/kg) was given 30 min after spiperone (0.3 mg/kg). Catalepsy was scored for 3 h at 30-min intervals. Groups consisted of 8 rats each.

2.7. Muscle tone

The method consists in measurement of the tone of extensor and flexor muscles of the hindlimb (according to Kolasiewicz et al., 1987).

2.8. Statistical analysis

The data were analyzed by 1- or 2-way analysis of variance (ANOVA) which, if significance was shown, was followed by Dunnett's test for post-hoc comparison with the control group. In the case of muscle tone, statistical evaluation was done with Student's *t*-test for dependent samples. The ED_{50} were calculated by the method of Litchfield and Wilcoxon (1949) from the Pharmacological Calculation System (PHARM/PCS), version 4.0.

2.9. Substances used

D-Amphetamine sulfate (SmithKline & French), apomorphine hydrochloride (Sandoz), caffeine pure (Polfa), CGP 37849 (D,L-E-amino-4-methyl-5-phosphono-3-pentenoic acid, Ciba Geigy), cocaine hydrochloride (Merck), EMD 57445 ((*S*)-(−)-5-[4-hydroxy-4-(3,4-benzodioxol-5-yl)-piperidin-1-ylmethyl]-3-(4-methoxyphenyl)-oxazolidin-2-one)(Merck), haloperidol (Richter), (+)-MK-801 (hydrogen maleate, Research Biochemicals), morphine (hydrochloride, Polfa), quinpirole hydrochloride (Lilly), (+)-SKF 38393 (Research Biochemicals), rimcazole (dihydrochloride, Burroughs Wellcome), spiperone hydrochloride (Research Biochemicals).

3. Results

3.1. Locomotor activity in rats and mice

EMD 57445 in doses of 3 and 10 mg/kg markedly decreased the locomotor activity of rats; a dose of 1 mg/kg was inactive (Fig. 1).

Rimcazole significantly decreased locomotor activity only at a high dose (40 mg/kg); decreases in locomotor activity after doses of 10 and 20 mg/kg were insignificant (Fig. 1).

The locomotor activity of mice was decreased only by the highest dose (10 mg/kg) of EMD 57445 used (294.7 ± 14.0 vs. 48.9 ± 6.2 as a control, i.e., to about 17%); other doses (1 and 3 mg/kg) of the compound, as well as rimcazole (5, 10 and 20 mg/kg), were ineffective.

3.2. D-Amphetamine-induced locomotor hyperactivity in rats

EMD 57445 (1, 3 and 10 mg/kg) dose-dependently attenuated the D-amphetamine-induced locomotor hyperactivity in rats (Fig. 2).

Rimcazole (5 and 10 mg/kg) markedly potentiated the effect of D-amphetamine; its dose of 20 mg/kg was ineffective (Fig. 2).

3.3. D-Amphetamine-induced stereotypy in rats

EMD 57445 (3 mg/kg) slightly attenuated the stereotyped behaviour induced by D-amphetamine (2.5 mg/kg); when used at a dose of 10 mg/kg, the drug completely counteracted the stereotypy. EMD 57445 (1 mg/kg) was inactive (Table 1).

Rimcazole (5 and 10 mg/kg) increased and prolonged the D-amphetamine-induced stereotypy; its dose of 20 mg/kg produced a weaker effect (Table 1).

3.4. Apomorphine-induced locomotor hyperactivity in rats

EMD 57445 (1, 3 and 10 mg/kg) markedly and dose-dependently decreased the apomorphine (0.3 mg/kg)-induced hyperactivity in rats (Fig. 3).

Rimcazole (5 mg/kg) increased the hyperactivity induced by apomorphine; its doses of 10 and 20 mg/kg were ineffective (Fig. 3).

3.5. Apomorphine-induced stereotypy in rats

EMD 57445 (3 and 10 mg/kg) decreased or completely counteracted, respectively, the stereotypy induced by apomorphine (0.5 mg/kg); the decrease after a dose of 1 mg/kg was weaker and insignificant (except for 1 time point) (Table 2).

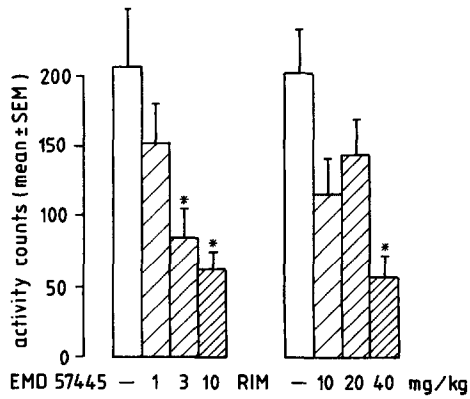

Fig. 1. The effect of EMD 57445 or rimcazole (RIM) on locomotor activity in rats. Results are expressed as means ± S.E.M. (after 1 h); $n = 8$. Significance values (* $P < 0.001$) are relative to controls (Dunnett's test).

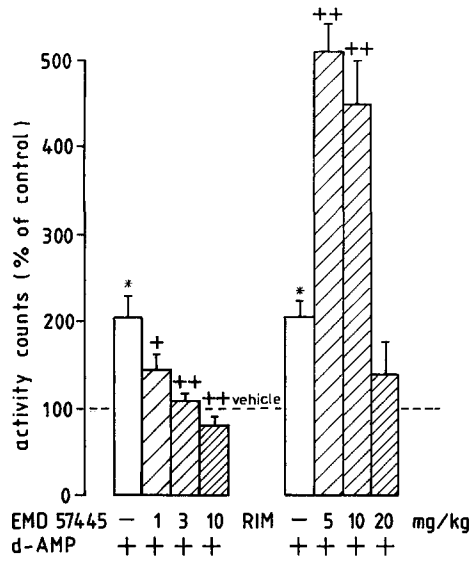

Fig. 2. The effect of EMD 57445 or rimcazole (RIM) on D-amphetamine (AMP, 0.5 mg/kg s.c.)-induced locomotor hyperactivity in rats. Results are expressed as percentages of control (mean ± S.E.M.) after 1 h; $n = 8$. Significance values are relative to adequate controls; * $P < 0.001$ vs. vehicle; + $P < 0.05$; ++ $P < 0.001$ vs. AMP (Dunnett's test).

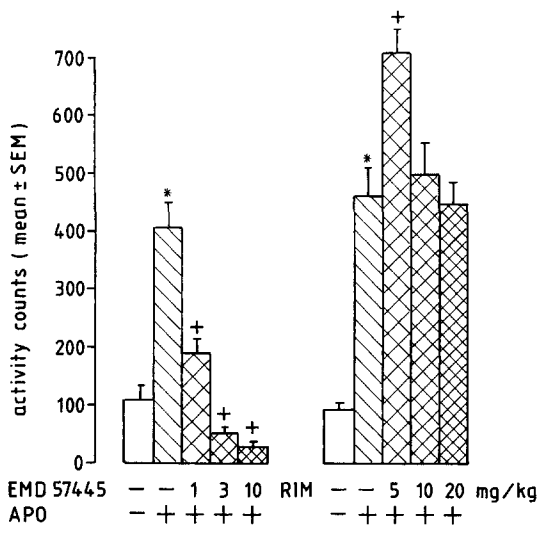

Fig. 3. The effect of EMD 57445 or rimcazole (RIM) on apomorphine (APO, 0.3 mg/kg s.c.)-induced locomotor hyperactivity in rats. Results are expressed as means ± S.E.M. ($n = 8$). Significance values are relative to adequate control * $P < 0.001$ vs. vehicle; + $P < 0.001$ vs. APO (Dunnett's test).

Rimcazole (5 and 10 mg/kg) did not modify the apomorphine-induced stereotypy; only its dose of 20 mg/kg slightly prolonged the effect of apomorphine (Table 2).

3.6. Apomorphine-induced aggression in rats

EMD 57445, as well as rimcazole, dose-dependently decreased the apomorphine (10 mg/kg)-induced aggression in rats; their ED_{50} values were 8.35 (1.37–50.78) and 14.85 (11.73–18.80) mg/kg, respectively.

3.7. Apomorphine-induced climbing in mice

EMD 57445, as well as rimcazole, dose-dependently inhibited the climbing behaviour induced in mice by apomorphine (0.75 mg/kg). Their ED_{50} values were 0.63 (0.27–1.43) and 13.47 (7.69–23.57) mg/kg, respectively.

3.8. SKF 38393-induced grooming in rats

EMD 57445 (10 mg/kg) decreased the time of grooming induced in rats by SKF 38393 (10 mg/kg); the time was 49.8 ± 5.0 ($P < 0.001$) vs. 175.4 ± 12.0 (SKF 38393 control). The doses of 1 and 3 mg/kg were ineffective (data not shown).

Rimcazole (5, 10 and 20 mg/kg) did not modify the SKF 38393-induced grooming (data not shown).

3.9. Quinpirole-induced locomotor hyperactivity in rats

EMD 57445 (1, 3 and 10 mg/kg) dose-dependently decreased the hyperactivity induced by quinpirole (0.3 mg/kg) in rats (Fig. 4).

Rimcazole (5 and 10 mg/kg) showed an opposite effect, i.e., it enhanced the effect of quinpirole; its dose of 20 mg/kg was ineffective (Fig. 4).

Table 1
The effects of EMD 57445 and rimcazole on the stereotypy induced by D-amphetamine (AMP, 2.5 mg/kg) in rats

Drug and dose (mg/kg)	Stereotypy score after					
	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle + AMP	1.3 ± 0.3	2.0 ± 0.0	2.0 ± 0.0	1.7 ± 0.2	1.0 ± 0.3	1.0 ± 0.3
EMD 57445 1 + AMP	0.8 ± 0.4	1.5 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	0.8 ± 0.3	0.5 ± 0.2
EMD 57445 3 + AMP	0.3 ± 0.2	1.2 ± 0.3	1.3 ± 0.2	0.7 ± 0.3	0.3 ± 0.2 [a]	0.0 ± 0.0 [a]
EMD 57445 10 + AMP	0.0 ± 0.0 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [a]
Vehicle + AMP	1.2 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	1.6 ± 0.2	1.0 ± 0.3	0.8 ± 0.3
RIM 5 + AMP	1.0 ± 0.0	1.7 ± 0.2	1.5 ± 0.2	2.7 ± 0.2 [b]	2.7 ± 0.2 [b]	2.3 ± 0.4 [b]
RIM 10 + AMP	1.3 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	2.5 ± 0.3 [a]	3.0 ± 0.0 [b]	2.3 ± 0.2 [b]
RIM 20 + AMP	1.2 ± 0.2	1.5 ± 0.2 [a]	1.2 ± 0.2 [b]	2.0 ± 0.2	2.0 ± 0.2 [a]	2.5 ± 0.2 [b]

EMD 57445 (p.o.) or RIM (i.p.) was given 0.5 h before AMP (s.c.). Results are expressed as means ± S.E.M. ($n = 8$). Significance values are relative to AMP control; [a] $P < 0.05$, [b] $P < 0.001$ (Dunnett's test).

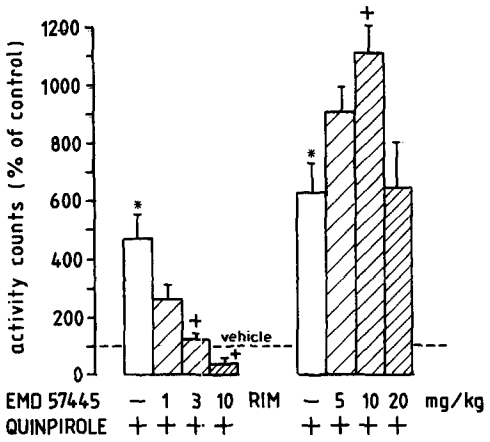

Fig. 4. The effect of EMD 57445 or rimcazole (RIM) on quinpirole (0.3 mg/kg s.c.)-induced locomotor hyperactivity in rats. Results are expressed as percentages of control (mean ± S.E.M.) after 2 h; $n = 8$. Significance values are relative to adequate controls: * $P < 0.001$ vs. vehicle; + $P < 0.001$ vs. quinpirole (Dunnett's test).

3.10. MK-801-induced locomotor hyperactivity in rats

EMD 57445 (10 mg/kg) decreased the locomotor hyperactivity induced by MK-801 (0.2 mg/kg) in rats to 425.0 ± 41.6, $P < 0.001$ (vs. MK-801 control, 904.4 ± 117.6), i.e., by about 53%. Its doses of 1 and 3 mg/kg were ineffective (data not shown).

Rimcazole (10 and 20 mg/kg) did not modify the effect of MK-801 in this model (data not shown).

3.11. CGP 37849-induced locomotor hyperactivity in rats

EMD 57445 (10 mg/kg) decreased the effect of CGP 37393 (20 mg/kg) in the locomotor activity test (117.1 ± 24.6, $P < 0.001$, vs. 472.1 ± 61.3 with CGP 37849 alone, i.e., by about 75%). Its doses of 1 and 3 mg/kg were ineffective (data not shown).

Rimcazole (10 mg/kg) increased the CGP 37849-induced locomotor hyperactivity (414.0 ± 29.6, $P < 0.001$) and, at 20 mg/kg, it evoked a decrease (183.1 ± 22.7, $P < 0.05$). The control value (CGP 37849 alone) was 267.3 ± 16.1.

3.12. Cocaine-induced locomotor hyperactivity in rats and mice

EMD 57445 (1, 3 and 10 mg/kg) decreased the cocaine (20 mg/kg)-induced hyperactivity in rats (Fig. 5).

Rimcazole (5, 10 and 20 mg/kg) dose-dependently increased the effect of cocaine in this test (Fig. 5).

In mice, the hyperactivity induced by cocaine (16 mg/kg) (519.1 ± 38.2, $P < 0.001$ vs. vehicle) was decreased by EMD 57445 (1, 3 and 10 mg/kg) to a value of 280.9 ± 39.9, 141.5 ± 6.6 and 41.8 ± 6.9, respectively ($P < 0.001$). Rimcazole (5, 10 and 20 mg/kg) was inactive in this test (data not shown).

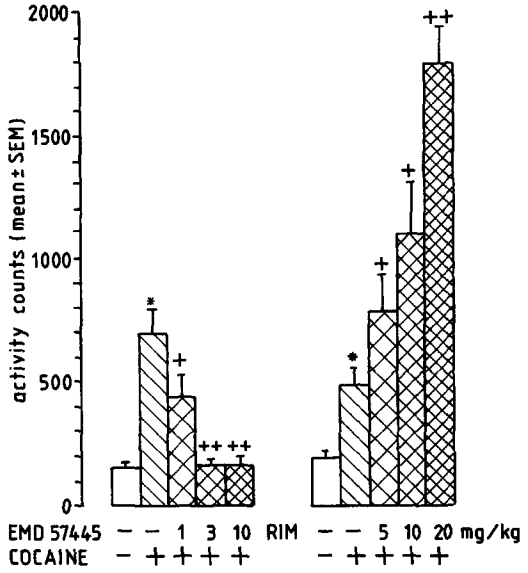

Fig. 5. The effect of EMD 57445 or rimcazole (RIM) on cocaine (20 mg/kg i.p.)-induced locomotor hyperactivity in rats. Results are expressed as means ± S.E.M. after 1 h; $n = 8$. Significance values are relative to adequate controls: * $P < 0.001$ vs. vehicle; + $P < 0.05$; ++ $P < 0.001$ vs. cocaine (Dunnett's test).

3.13. Morphine-induced locomotor hyperactivity in mice

The locomotor hyperactivity induced by morphine (20 mg/kg) was markedly decreased by all the doses (1–10 mg/kg) of EMD 57445 tested (Fig. 6).

Rimcazole (5, 10 and 20 mg/kg) did not affect the above effect of morphine significantly (data not shown).

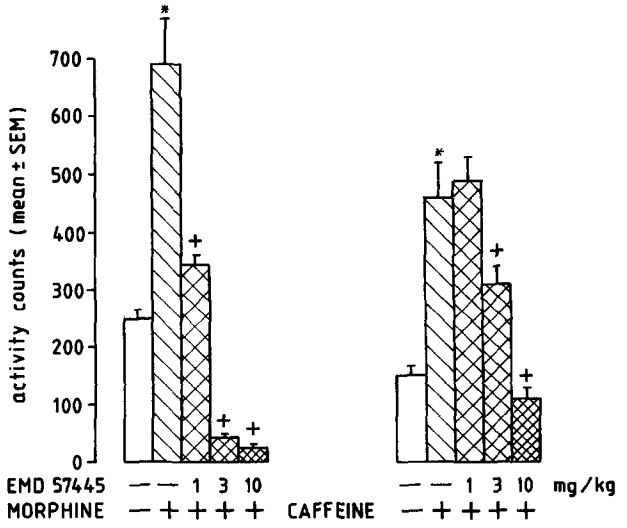

Fig. 6. The effect of EMD 57445 on morphine (20 mg/kg i.p.) (mice) or caffeine (20 mg/kg i.p.) (rats)-induced locomotor hyperactivity. Results are expressed as means ± S.E.M. after 1 h; $n = 8$. Significance values are relative to adequate controls: * $P < 0.001$ vs. vehicle; + $P < 0.001$ vs. morphine (Dunnett's test).

Table 2
The effects of EMD 57445 and rimcazole on the stereotypy induced by apomorphine (APO, 0.5 mg/kg) in rats

Drug and dose (mg/kg)	Stereotypy score after					
	10 min	20 min	30 min	40 min	50 min	60 min
Vehicle + APO	1.5 ± 0.2	2.2 ± 0.3	2.5 ± 0.3	1.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
EMD 57445 1 + APO	1.3 ± 0.4	1.5 ± 0.3	1.5 ± 0.2	0.5 ± 0.2 [a]	0.0 ± 0.0	0.0 ± 0.0
EMD 57445 3 + APO	0.2 ± 0.2 [a]	0.7 ± 0.3 [a]	0.7 ± 0.3 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0	0.0 ± 0.0
EMD 57445 10 + APO	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.3 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0	0.0 ± 0.0
Vehicle + APO	1.2 ± 0.2	1.5 ± 0.3	1.5 ± 0.3	1.2 ± 0.2	0.3 ± 0.2	0.0 ± 0.0
RIM 5 + APO	1.2 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.2 ± 0.2	0.5 ± 0.2	0.3 ± 0.2
RIM 10 + APO	0.8 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.0
RIM 20 + APO	0.5 ± 0.2	1.6 ± 0.3	1.6 ± 0.3	2.3 ± 0.3 [a]	2.4 ± 0.3 [a]	1.9 ± 0.3 [a]

EMD 57445 (p.o.) or RIM (i.p.) was given 0.5 h before APO (s.c.). Results are expressed as means ± S.E.M. ($n = 8$). Significance values are relative to APO control; [a] $P < 0.001$ (Dunnett's test).

3.14. Caffeine-induced locomotor hyperactivity in rats

EMD 57445 (3 and 10 mg/kg) decreased the hyperactivity induced by caffeine (20 mg/kg); its dose of 1 mg/kg was inactive (Fig. 6).

Rimcazole (5, 10 and 20 mg/kg) did not change the effect of caffeine in this test (data not shown).

3.15. Catalepsy in rats

EMD 57445 (1 or 10 mg/kg) did not itself induce catalepsy in rats. Only its high dose (20 mg/kg) showed a slight cataleptogenic effect (maximal value was 1 point) (data not shown).

Rimcazole (up to a dose of 40 mg/kg) did not induce catalepsy in rats (data not shown).

The spiperone (0.3 mg/kg)-induced catalepsy was not changed by EMD 57445 (1 and 10 mg/kg); rimcazole (5 and 10 mg/kg) attenuated the cataleptogenic effect of spiperone (spiperone, 38.0 ± 1.7; spiperone + rimcazole 5 mg/kg, 24.9 ± 2.1, $P < 0.001$; spiperone + rimcazole 10 mg/kg, 18.1 ± 2.6, $P < 0.001$).

3.16. Muscle tone

In contrast to haloperidol (1 mg/kg), which increased the tone of extensor or flexor muscles in rats, EMD 57445 (1 and 10 mg/kg) did not influence the tone of either group of muscles (data not shown).

4. Discussion

Our results indicate that EMD 57445 diminishes locomotor activity in rats and mice. Rimcazole acts likewise, but a clear effect was observed only at a high dose (40 mg/kg).

EMD 57445 antagonized both D-amphetamine effects studied, i.e., the locomotor hyperactivity and stereotypy, in rats. In this respect, it differs clearly from rimcazole which increases both D-amphetamine-induced effects. EMD 57445 antagonizes all the effects of apomorphine we now studied: locomotor hyperactivity, stereotypy, aggression and climbing (the latter one in mice). The EMD 57445-induced inhibition of apomorphine stereotypy and climbing (ED_{50} = 1.2 mg/kg p.o.) was reported by Bartoszyk et al. (1996). The latter authors also observed reduction of the apomorphine-induced contralateral turning behaviour by EMD 57445 (Bartoszyk et al., 1996).

Rimcazole increased the apomorphine hyperlocomotion (at one low dose only), had no effect on the stereotypy (or slightly prolonged it at the highest dose) and inhibited the aggression and climbing. The ED_{50} of EMD 57445 in the 2 latter tests were about 2 and 20 times lower, respectively, than those of rimcazole. Ferris et al. (1982) also found antagonism of rimcazole towards the apomorphine aggression in rats ($ED_{50} = 48 \pm 6.9$ mg/kg p.o.) and the climbing in mice ($ED_{50} = 19 \pm 4$ mg/kg i.p.), but no influence on the stereotypy in rats.

EMD 57445 antagonized the effects induced by SKF 38393, a dopamine D_1 receptor agonist (grooming behaviour) and by quinpirole, a dopamine D_2/D_3 receptor agonist (hyperlocomotion). Rimcazole did not change the SKF 38393-evoked effect and increased the effect of quinpirole.

The NMDA receptor antagonists, MK-801 (non-competitive) and CGP 37849 (competitive) induce locomotor hyperactivity mainly via indirect activation of the dopamine system (Clineschmidt et al., 1982; Rao et al., 1990; Maj et al., 1991, 1993b). EMD 57445 inhibited (in both cases, at the highest dose) the effects of MK-801 and CGP 37849. Rimcazole did not influence the effect of MK-801 and increased or decreased (depending on the dose) the action of CGP 37849.

We have also studied the influence of EMD 57445 on the locomotor hyperactivity induced by other behaviour stimulants, cocaine, morphine and caffeine, in rats and mice. The effect of any of the 3 stimulants was clearly antagonized by EMD 57445, as it was by spiperone (own unpublished data), a drug with high affinity for the dopamine receptor but without affinity for the σ receptor.

Rimcazole increased the effect of cocaine in rats (but not in mice), decreased that of caffeine (rats) and did not change the effect of morphine (mice).

Our results, in particular the antagonism towards D-amphetamine, indicate that EMD 57445 has an antidopaminergic, neuroleptic-like activity. Some other evidence for such an activity of EMD 57445 emerges from the inhibition of avoidance behaviour ($ED_{50} = 5$ mg/kg) (Bartoszyk et al., 1996). However, in contrast to neuroleptics, the drug does not evoke catalepsy (the present results; Bartoszyk et al., 1996, in doses of up to 1000 mg/kg p.o.). Moreover, it does not change muscle tone, whereas haloperidol (and other neuroleptics) increases it.

EMD 57445 shows a high affinity for σ receptors ($IC_{50} = 6$ nM, for comparison, haloperidol $IC_{50} = 1$ nM) and no affinity for dopamine receptors (D_1, $IC_{50} > 10000$ nM; D_2, $IC_{50} = 5200$ nM; haloperidol: D_1, $IC_{50} = 130$ nM; D_2, $IC_{50} = 4$ nM) (Bartoszyk et al., 1996). EMD 57445 has a high selectivity, as it does not bind to a number of other receptors, such as 5-HT_1, 5-HT_2, 5-HT_3, α-$_1$, α-$_2$, muscarinic, GABA, excitatory amino acid, mi, kappa, delta (Bartoszyk et al., 1996). It may, therefore, be concluded that the functional antidopaminergic effects of EMD 57445 are mediated by its action on σ receptors (binding sites).

The antidopaminergic effects of EMD 57445 allow us to expect its clinical, antipsychotic activity. The failure to induce catalepsy indicates that EMD 57445 may be devoid of extrapyramidal side-effects in humans. Moreover, in contrast to haloperidol, the drug given chronically does not induce supersensitization of dopamine D_2 receptors (no increase in the apomorphine-induced stereotypy) (Bartoszyk et al., 1996)

It is noteworthy that EMD 57445 was able to antagonize the behavioural stimulation induced by all the drugs used in the present study, which have different pharmacological profiles. It could be supposed that the drug may be effective, not only in psychosis, but also in other states of excitation, e.g., mania or aggression, in man.

As mentioned above, there exist some distinct differences between the two σ receptor ligands studied, EMD 57445 and rimcazole. The most important difference is that EMD 57445 decreases, while rimcazole increases, locomotor hyperactivity and stereotypy, both these effects being induced by D-amphetamine (according to Ceci et al., 1988, rimcazole does not change the D-amphetamine hyperlocomotion induced by a high dose of the latter substance). The locomotor hyperactivity induced by quinpirole and cocaine is also blocked by EMD 57445. All three behavioural stimulants act via a dopamine mechanism. The binding data indicate that rimcazole has an affinity for σ receptors in the rat brain lower than that of EMD 57445, as well as lower selectivity (Largent et al., 1988; Tam et al., 1992), but these effects do not resolve the pharmacological differences mentioned above. At the moment, no explanation can be offered for these differences.

Our results indicate that EMD 57445, a selective σ receptor ligand, has functional antidopaminergic activities in a number of tests used here. These activities become evident when the dopamine system is stimulated. In some tests, an antistimulant effect of EMD 57445 is observed at doses which do not inhibit locomotion in normal animals.

EMD 57445 is also active in normal animals, e.g., it inhibits avoidance behaviour (Bartoszyk et al., 1996). On the other hand, EMD 57445 does not induce catalepsy, which is typical of dopamine receptor antagonists. The reason for the above differences is not clear.

It still remains unclear how the σ receptor/dopamine receptor interaction is triggered. It can only be speculated that this interaction proceeds via other systems, e.g., via the NMDA system. For example, EMD 57445 potentiates the convulsive effects of NMDA (own unpublished data).

The pharmacological profiles of EMD 57445 and rimcazole differ widely. It is worth adding that another, fairly selective σ receptor ligand, DTG (Weber et al., 1986), still shows a diverse pharmacological profile. In contrast to EMD 57445 (but like rimcazole), DTG increases the apomorphine- and quinpirole-induced hyperlocomotion, as well as the D-amphetamine stereotypy; it also shows an anticataleptic effect (on neuroleptic-induced catalepsy) and does not modify the D-amphetamine-induced hyperlocomotion (the latter effect differs from those of both EMD 57445 and rimcazole) (Maj et al., 1994).

The existing differences in pharmacological profile of σ receptor ligands (in particular these already discussed in the present paper) may stem from, e.g., dissimilarities in their affinity for the σ receptor subpopulations (σ_1 and σ_2), whose existence has been suggested (Quirion et al., 1992; Walker et al., 1992). However, quantitation of the differences in affinity for σ_1- and σ_2-receptors is still impossible due to a lack of selective ligands; moreover, their functional importance has not yet been assessed.

Like other data, our results do not allow us, as yet, to determine unequivocally whether EMD 57445 is a σ receptor agonist or antagonist. This concerns other σ ligands as well; for example DTG, which is assumed by some authors to have σ-agonistic activity, lowers body temperature (Bejanian et al., 1991) but the decrease is not inhibited by rimcazole, which is supposed to have a σ receptor-antagonistic activity (Ferris et al., 1986a). We would be tempted to ascribe an antagonistic action to EMD 57445, but – as shown by the present results – it differs markedly from rimcazole in some respects. Thus, definition of a given σ receptor ligand as agonist or antagonist is still a matter of speculation at the present stage of knowledge.

EMD 57445 may be classified as a functional antidopaminergic agent, neuroleptic-like, but without capacity to induce catalepsy. This is an important difference from typical neuroleptics which induce a number of neuroleptic-like antidopaminergic effects but also catalepsy. EMD 57445 also differs from such atypical neuroleptics, as clozapine, which shows no affinity to σ

receptors (Bartoszyk et al., 1996). EMD 57445 may, therefore, represent a new class of potential antipsychotic drugs.

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